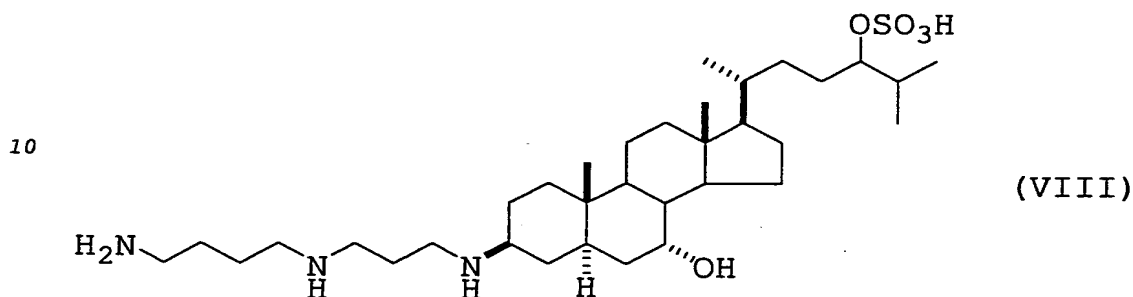## DESCRIPTION

METHOD FOR PRODUCING 5α-PREGNANE DERIVATIVE

Technical Field

The present invention relates to production methods of

5 5α-pregnane derivatives useful as synthetic intermediates for squalamine.

Background Art

Squalamine is a compound represented by the formula (VIII):

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(VIII)

, which has been reported to show strong antibacterial activity against Gram-positive bacteria, Gram-negative bacteria, fungi and the like, as well as anticancer activity, and is drawing attention as a new antibiotic.

15 Conventionally, squalamine is extracted from the liver of dogfish. In view of its extremely low content of 0.001-0.002 wt% and poor extraction efficiency, however, various chemical synthetic methods have been studied. Particularly, (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one represented by the

20 formula (VI):

(VI)
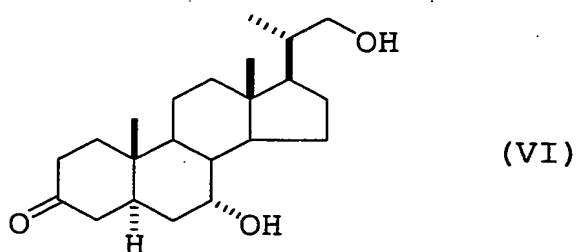
(WO01/79255, Organic Letters, Vol. 2, p. 2921 (2000)) and (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-3-one represented by the formula (IX):

(IX)
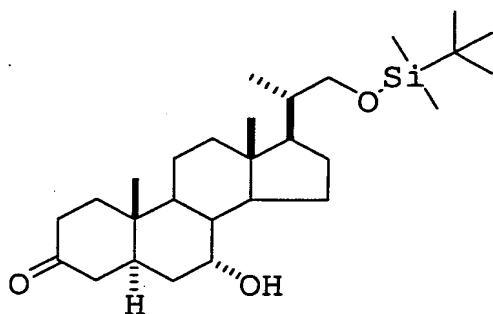
(WO03/51904) are known to be useful intermediates that can be converted to squalamine comparatively in a few steps.

Conventionally, as production methods of (20S)-7α,21-
dihydroxy-20-methyl-5α-pregn-3-one, a method (WO01/79255) including subjecting (20S)-7α,21-dihydroxy-20-methylpregn-4-en-3-one to what is called the Birch reduction using not less than 30 equivalents of metal lithium in liquid ammonia with the aim of stereoselective reduction to an α form in the 5-position, a method (WO02/20552) including subjecting (20S)-7α,21-dihydroxy-20-methylpregna-1,4-dien-3-one to the Birch reduction using 10 equivalents of metal lithium in liquid ammonia, and the like have been developed.

In addition, as a production method of (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-3-one, a method (WO03/51904) including reducing (20S)-7α,21-dihydroxy-20-methylpregna-1,4-dien-3-one in the same manner as in the above to give (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one, and then protecting the hydroxyl group at 21-position of the compound with a tert-butyldimethylsilyl group is known.

Disclosure of the Invention

However, the yield of the above-mentioned method is 71% at the highest and, in consideration of the fact that the pregnane derivative is an expensive raw material, the method cannot be considered a preferable production method but has a room for improvement before its industrial practice.

Therefore, an object of the present invention is to provide a method of efficiently producing (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one useful as an synthetic

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intermediate for squalamine and a (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one derivative wherein the hydroxyl group(s) at the 21-position and/or the 7-position are/is protected with protecting group(s), which comprises stereoselectively

reducing a (20S)-7α,21-dihydroxy-20-methylpregna-1,4-dien-3-one derivative or a (20S)-7α,21-dihydroxy-20-methylpregn-4-en-3-one derivative to a 5α form, and then, where necessary, eliminating the hydroxyl-protecting group(s) of the 5α form.

In the aforementioned conventional reaction, a ketone derivative stereoselectively converted to a 5α form is obtained from an unsaturated ketone having at least the carbon-carbon double bond at 4- and 5-positions as a raw compound, which corresponds to what is called the partial reduction where an unsaturated ketone is converted to a saturated ketone. According to the conventional reaction, however, it has been clarified that the saturated ketone is further reduced to give an alcohol form due to the side reaction. To suppress such side reaction, it is important that the reaction be carried out using a reducing agent in an amount close to the equivalent amount, which is necessary for the partial reduction. However, in the conventional reaction, a large excess of metal lithium is used.

The present inventors have conducted intensive studies of the reason for low yields by conventional methods and found that since a hydroxyl group is present at the 21-position of the raw material pregnane derivative, namely, since metal lithium, which is a reducing agent, is decomposed due to the highly reactive primary hydroxyl group present at the 21-position to lose reducing ability, use of excess metal lithium is unavoidable, and that since the primary hydroxyl group also acts as a proton donor during the reduction reaction, which in turn further promotes the reaction, an alcohol form is by-produced.

Based on such finding, a reduction reaction was carried out using a compound wherein the hydroxyl group at the 21-

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position is protected as a raw compound. As a result, the decomposition of the reducing agent due to the hydroxyl group and the action as a proton donor were suppressed. Furthermore, it was newly found that, in this reaction, the reduction reaction of the carbon-carbon double bond at the 4,5-positions of a (20S)-7α,21-dihydroxy-20-methylpregna-1,4-dien-3-one derivative proceeds significantly faster than the reduction reaction of the carbon-carbon double bond at the 1,2-positions. Based on such finding, it was found that a reduction reaction with a reducing agent in a reduced amount sufficient to mostly complete the reduction of the carbon-carbon double bond at the 4,5-positions but partly leaving the carbon-carbon double bond at the 1,2-positions affords a ketone derivative stereoselectively converted to a 5α form as a mixture of a 5α-saturated ketone form and a 5α-1-en-3-one form in a high yield of a total of the both compounds.

Moreover, the present inventors have conducted intensive studies and found that, by reducing, after deprotection as necessary of each of the hydroxyl-protecting groups that the mixture of the 5α-saturated ketone form and the 5α-1-en-3-one form have, the carbon-carbon double bond at the 1,2-positions of 5α-1-en-3-one form therein, the objective 5α-saturated ketone form can be produced in a high yield while significantly suppressing the reduction of saturated ketone. As a result, the amount of the reducing agent to be used can be decreased, the side reaction can be suppressed and the yield of the objective 5α-pregnane derivative can be increased, thus solving the problems of the conventional methods.

Accordingly, the present invention provides the following.

(1) A method for producing a mixture of a 5α-pregnane derivative represented by the formula (II):

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(II)
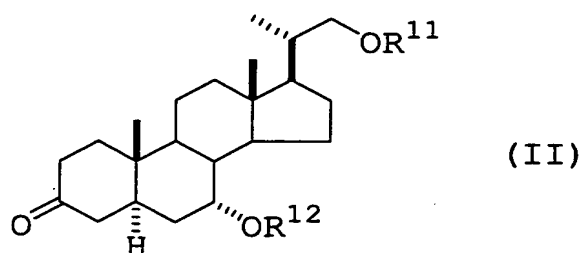
wherein R^{11} and R^{12} are each independently a hydrogen atom or a hydroxyl-protecting group (hereinafter sometimes to be referred to as compound (II) in the present specification) and

5 a 5α-pregnane derivative represented by the formula (III):

(III)
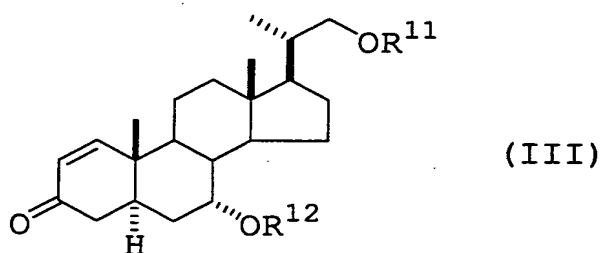
wherein R^{11} and R^{12} are as defined above (hereinafter sometimes to be referred to as compound (III) in the present specification), which comprises reacting a pregnane derivative

10 represented by the formula (I):

(I)
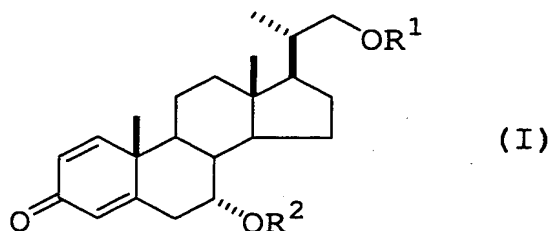
wherein R^1 is a hydroxyl-protecting group and R^2 is a hydrogen atom or a hydroxyl-protecting group (hereinafter sometimes to be referred to as compound (I) in the present specification),

15 with a metal selected from alkali metals and alkaline earth metals in the presence of a proton donor and an amine and/or ammonia.

(2) The method of the above-mentioned (1), wherein R^2 and R^{12} are hydrogen atoms.

20 (3) The method of the above-mentioned (2), wherein R^1 and R^{11} are tri-substituted silyl groups having three, same or

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different, substituents selected from the group consisting of
an alkyl group optionally having substituent(s), an aryl group
optionally having substituent(s), an alkoxyl group optionally
having substituent(s) and an aryloxy group optionally having
substituent(s).

(4) The method of the above-mentioned (3), wherein R^1 and R^{11}
are tert-butyldimethylsilyl groups.

(5) The method of any one of the above-mentioned (1)-(4),
wherein the metal is an alkali metal.

(6) The method of the above-mentioned (5), wherein the alkali
metal is lithium.

(7) A method for producing a mixture of (20S)-7α,21-dihydroxy-
20-methyl-5α-pregn-3-one represented by the formula (VI):

(VI)
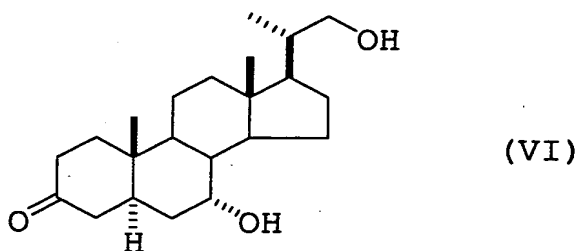
(hereinafter sometimes to be referred to as compound (VI) in
the present specification) and (20S)-7α,21-dihydroxy-20-
methyl-5α-pregn-1-en-3-one represented by the formula (VII):

(VII)
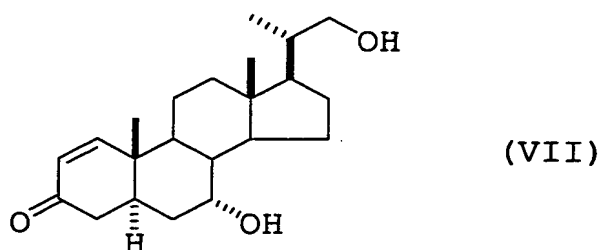
(hereinafter sometimes to be referred to as compound (VII) in
the present specification), which comprises the steps of
(a) reacting compound (I) with a metal selected from alkali
metals and alkaline earth metals in the presence of a proton
donor and an amine and/or ammonia to give a mixture of a 5α-
pregnane derivative represented by the formula (IV):

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(IV)
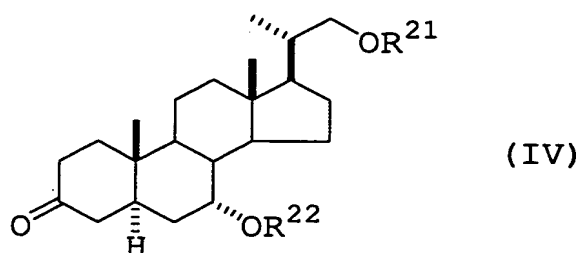
wherein R^{21} is a hydroxyl-protecting group and R^{22} is a hydrogen atom or a hydroxyl-protecting group (hereinafter sometimes to be referred to as compound (IV) in the present specification) and a 5α-pregnane derivative represented by the formula (V):

(V)
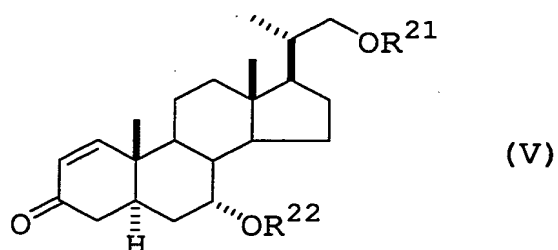
wherein R^{21} and R^{22} are as defined above (hereinafter sometimes to be referred to as compound (V) in the present specification); and

(b) eliminating the hydroxyl-protecting groups of the mixture obtained by the aforementioned step.

(8) The method of the above-mentioned (7), wherein R^2 and R^{22} are hydrogen atoms.

(9) The method of the above-mentioned (8), wherein R^1 and R^{21} are tri-substituted silyl groups defined above.

(10) The method of the above-mentioned (9), wherein R^1 and R^{21} are tert-butyldimethylsilyl groups.

(11) The method of any one of the above-mentioned (7)-(10), wherein the metal is an alkali metal.

(12) The method of the above-mentioned (11), wherein the alkali metal is lithium.

According to the present invention, by using a compound wherein the hydroxyl group at the 21-position is protected as a raw compound for producing a 5α-pregnane derivative by stereoselectively reducing a pregnana-1,4-diene derivative,

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the reaction can be carried out using a decreased amount of
the reducing agent. In addition, since the method of the
present invention involves a reduction using a smaller amount
of a reducing agent than conventional cases, production of an
alcohol form due to the side reaction can be suppressed.
Moreover, by a 2-step reduction treatment comprising further
reducing only the carbon-carbon double bond at the 1,2-
positions of compound (V) or compound (VII) remaining in the
resultant product without being completely reduced during the
reduction reaction, a 5α-pregnane derivative useful as a
synthetic intermediate for squalamine can be produced in a
high yield. According to the method of the present invention,
excessive use of a reducing agent as in the conventional
methods becomes unnecessary, which in turn obliterates side
reactions and provides a beneficial economical effect.

Best Mode for Embodying the Invention

1. Explanation of symbols

In the above-mentioned formulas, the hydroxyl-protecting
group represented by R^1, R^2, R^{11}, R^{12}, R^{21} or R^{22} may be any as
long as it acts as a hydroxyl-protecting group and, for
example, an alkyl group optionally having substituent(s); an
acyl group optionally having substituent(s) (e.g., a formyl
group, an alkylcarbonyl group optionally having
substituent(s), an alkenylcarbonyl group optionally having
substituent(s), an arylcarbonyl group optionally having
substituent(s) etc.); an alkoxycarbonyl group optionally
having substituent(s); an aryloxycarbonyl group optionally
having substituent(s); a carbamoyl group (e.g., a carbamoyl
group wherein the nitrogen atom is optionally substituted by
an alkyl group optionally having substituent(s) or an aryl
group optionally having substituent(s)); a tri-substituted
silyl group (the tri-substituted silyl group has three, same
or different, substituents selected from the group consisting
of an alkyl group optionally having substituent(s), an aryl
group optionally having substituent(s), an alkoxyl group

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optionally having substituent(s) and an aryloxy group optionally having substituent(s)); and the like can be mentioned.

The alkyl group as the hydroxyl-protecting group represented by R^1, R^2, R^{11}, R^{12}, R^{21} or R^{22}; the alkyl group as a part of the acyl group and the alkyl group as a substituent that the acyl group optionally has; the alkyl group as a part of the alkoxycarbonyl group; the alkyl group as a substituent that the carbamoyl group optionally has; the alkyl group that the tri-substituted silyl group has, the alkyl group as a part of the alkoxyl group that the tri-substituted silyl group has, and the alkyl group as a substituent that the aryl group and aryloxy group that the tri-substituted silyl group has optionally have, may be linear, branched or cyclic, and preferably has 1 to 12, more preferably 1 to 8, carbon atoms. As such alkyl group, for example, methyl group, ethyl group, propyl group, isopropyl group, butyl group, isobutyl group, tert-butyl group, hexyl group, octyl group, dodecyl group, cyclopentyl group, cyclohexyl group and the like can be mentioned.

The above-mentioned alkyl group optionally has substituent(s). While the number of substituents is not particularly limited, 1 to 6 is preferable, and when the number is two or more, the substituents may be the same or different. As such substituent, for example, an aryl group having 6 to 12, preferably 6 to 10, carbon atoms, which optionally has substituent(s), such as phenyl group, tolyl group, methoxyphenyl group, nitrophenyl group, naphthyl group, fluorenyl group and the like; an alkenyl group having 2 to 12, preferably 2 to 10, carbon atoms such as vinyl group and the like, which optionally has substituent(s); a linear, branched or cyclic alkoxyl group having 1 to 12, preferably 1 to 8, carbon atoms (the alkoxyl group may form a ring structure (e.g., tetrahydropyran ring, tetrahydrofuran ring etc.) together with an alkyl group which is a hydroxyl-protecting

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group) such as methoxy group, ethoxy group, propoxy group, isopropoxy group, butoxy group, isobutoxy group, tert-butoxy group, hexyloxy group, octyloxy group, dodecyloxy group, cyclopentyloxy group, cyclohexyloxy group and the like; an aralkyloxy group having 7 to 12, preferably 7 to 11, carbon atoms such as benzyloxy group and the like; an alkenyloxy group having 2 to 12, preferably 2 to 8, carbon atoms such as allyloxy group and the like; an aryloxy group having 6 to 12, preferably 6 to 10, carbon atoms such as phenoxy group, naphthyloxy group and the like, which optionally has substituent(s); and the like can be mentioned.

The alkenyl group as a part of the acyl group as the hydroxyl-protecting group represented by R^1, R^2, R^{11}, R^{12}, R^{21} or R^{22}, and the alkenyl group as a substituent that the acyl group optionally has; the alkenyl group as a substituent that the aryloxycarbonyl group optionally has; and the alkenyl group as a substituent that the aryl group, alkoxyl group and aryloxy group that the tri-substituted silyl group has optionally have, may be linear, branched or cyclic, and preferably has 2 to 12, more preferably 2 to 8, carbon atoms. As such alkenyl group, for example, vinyl group, 1-methylvinyl group, 1-propenyl group, 1-octenyl group, 1-dodecenyl group, 1-cyclopentenyl group, 1-cyclohexenyl group and the like can be mentioned.

The above-mentioned alkenyl group optionally has substituent(s). While the number of substituents is not particularly limited, 1 to 6 is preferable, and when the number is two or more, the substituents may be the same or different. As such substituent, for example, an aryl group having 6 to 12, preferably 6 to 10, carbon atoms, which optionally has substituent(s), such as phenyl group, tolyl group, methoxyphenyl group, nitrophenyl group, naphthyl group, fluorenyl group and the like; a linear, branched or cyclic alkoxyl group having 1 to 12, preferably 1 to 8, carbon atoms such as methoxy group, ethoxy group, propoxy group, isopropoxy

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group, butoxy group, isobutoxy group, tert-butoxy group, hexyloxy group, octyloxy group, dodecyloxy group, cyclopentyloxy group, cyclohexyloxy group and the like; an aralkyloxy group having 7 to 12, preferably 7 to 11, carbon

5 atoms such as benzyloxy group and the like; an alkenyloxy group having 2 to 12, preferably 2 to 8, carbon atoms such as allyloxy group and the like; an aryloxy group having 6 to 12, preferably 6 to 10, carbon atoms such as phenoxy group, naphthyloxy group and the like, which optionally has

10 substituent(s); and the like can be mentioned.

The aryl group as a part of the acyl group as the hydroxyl-protecting group represented by R^1, R^2, R^{11}, R^{12}, R^{21} or R^{22}, and the aryl group as a substituent that the acyl group optionally has; the aryl group as a part of the

15 aryloxycarbonyl group and the aryl group as a substituent that the aryloxycarbonyl group optionally has; the aryl group as a substituent that the carbamoyl group optionally has; the aryl group that the tri-substituted silyl group has, the aryl group as a part of the aryloxyl group that the tri-substituted silyl

20 group has, and the aryl group as a substituent that the aryl group, alkoxyl group and aryloxy group that the tri-substituted silyl group has optionally have, preferably have 6 to 10 carbon atoms and, for example, phenyl group, naphthyl group and the like can be mentioned.

25 The above-mentioned aryl group optionally has substituent(s). While the number of substituents is not particularly limited, 1 to 6 is preferable, and when the number is two or more, the substituents may be the same or different. As such substituent, for example, a linear,

30 branched or cyclic alkyl group having 1 to 12, preferably 1 to 8, carbon atoms such as methyl group, ethyl group, propyl group, isopropyl group, butyl group, isobutyl group, tert-butyl group, hexyl group, octyl group, dodecyl group, cyclopentyl group, cyclohexyl group and the like; a linear,

35 branched or cyclic alkoxyl group having 1 to 12, preferably 1

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to 8, carbon atoms such as methoxy group, ethoxy group, propoxy group, isopropoxy group, butoxy group, isobutoxy group, tert-butoxy group, hexyloxy group, octyloxy group, dodecyloxy group, cyclopentyloxy group, cyclohexyloxy group and the like; a linear, branched or cyclic acyloxy group having 1 to 12, preferably 1 to 8, carbon atoms such as formyloxy group, acetyloxy group, propionyloxy group, butyryloxy group, isobutyryloxy group, valeryloxy group, isovaleryloxy group, pivaloyloxy group, hexanoyloxy group, octanoyloxy group, dodecanoyloxy group, cyclopentanecarbonyloxy group, cyclohexanecarbonyloxy group, benzoyloxy group, methoxybenzoyloxy group, nitrobenzoyloxy group and the like; nitro group; cyano group and the like can be mentioned.

Of the hydroxyl-protecting groups represented by R^1, R^2, R^{11}, R^{12}, R^{21} or R^{22}, specific examples of the alkyl group optionally having substituent(s) include methyl group, ethyl group, tert-butyl group, methoxymethyl group, tert-butoxymethyl group, benzyloxymethyl group, 2-tetrahydropyranyl group, 2-tetrahydrofuranyl group, 1-ethoxyethyl group, 1-benzyloxyethyl group, benzyl group, p-methoxybenzyl group, p-nitrobenzyl group, trityl group and the like, with preference given to methyl group, ethyl group, methoxymethyl group, 2-tetrahydropyranyl group, 2-tetrahydrofuranyl group and 1-ethoxyethyl group.

Of the hydroxyl-protecting groups represented by R^1, R^2, R^{11}, R^{12}, R^{21} or R^{22}, specific examples of the acyl group include formyl group, acetyl group, propionyl group, butyryl group, isobutyryl group, valeryl group, isovaleryl group, pivaloyl group, hexanoyl group, octanoyl group, dodecanoyl group, cyclopentanecarbonyl group, cyclohexanecarbonyl group, methoxyacetyl group, crotonoyl group, cinnamoyl group, phenylacetyl group, phenoxyacetyl group, benzoyl group, methoxybenzoyl group, nitrobenzoyl group and the like, with preference given to formyl group, acetyl group, propionyl

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group and pivaloyl group.

Of the hydroxyl-protecting groups represented by R^1, R^2, R^{11}, R^{12}, R^{21} or R^{22}, specific examples of the alkoxycarbonyl group optionally having substituent(s) include methoxycarbonyl group, ethoxycarbonyl group, propoxycarbonyl group, isopropoxycarbonyl group, butoxycarbonyl group, isobutoxycarbonyl group, tert-butoxycarbonyl group, hexyloxycarbonyl group, octyloxycarbonyl group, dodecyloxycarbonyl group, cyclopentyloxycarbonyl group, cyclohexyloxycarbonyl group, benzyloxycarbonyl group, p-methoxybenzyloxycarbonyl group, fluorenylmethoxycarbonyl group, p-nitrobenzyloxycarbonyl group, allyloxycarbonyl group and the like, with preference given to methoxycarbonyl group, ethoxycarbonyl group, isobutoxycarbonyl group and allyloxycarbonyl group.

Of the hydroxyl-protecting groups represented by R^1, R^2, R^{11}, R^{12}, R^{21} or R^{22}, specific examples of the aryloxycarbonyl group optionally having substituent(s) include phenoxycarbonyl group, p-nitrophenoxycarbonyl group and the like, with preference given to phenoxycarbonyl group.

Of the hydroxyl-protecting groups represented by R^1, R^2, R^{11}, R^{12}, R^{21} or R^{22}, specific examples of the carbamoyl group include carbamoyl group wherein any hydrogen atom that a nitrogen atom has is optionally substituted, for example, by a linear, branched or cyclic alkyl group having 1 to 12 carbon atoms such as methyl group, ethyl group, propyl group, isopropyl group, butyl group, isobutyl group, tert-butyl group, hexyl group, octyl group, dodecyl group, cyclopentyl group, cyclohexyl group and the like, an aralkyl group having 7 to 12 carbon atoms such as benzyl group and the like, an alkenyl group having 2 to 12 carbon atoms such as allyl group and the like or an aryl group having 6 to 10 carbon atoms, which optionally has substituent(s), such as phenyl group, methoxyphenyl group, naphthyl group and the like, and the like.

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Of the hydroxyl-protecting groups represented by R^1, R^2, R^{11}, R^{12}, R^{21} or R^{22}, specific examples of the tri-substituted silyl group include trimethylsilyl group, triethylsilyl group, triisopropylsilyl group, dimethylisopropylsilyl group,

5 diethylisopropylsilyl group, tert-butyldimethylsilyl group, tert-butyldiphenylsilyl group, tribenzylsilyl group, tert-butylmethoxyphenylsilyl group and the like, with preference given to tert-butyldimethylsilyl group, triethylsilyl group and triisopropylsilyl group, and more preference given to

10 tert-butyldimethylsilyl group.

As R^1, R^{11} or R^{21}, a tri-substituted silyl group is preferable, and a tert-butyldimethylsilyl group is more preferable.

Since the hydroxyl group at the 7-position in compound

15 (I) is slow in reaction with a metal reducing agent due to steric hindrance and does not adversely influence the reaction, it may or may not be protected. However, since introductory reaction of the protecting group can be omitted, it is preferably not protected. Therefore, as R^2, R^{12} and R^{22},

20 hydrogen atoms are preferable.

2. Reduction method and reaction conditions

The method for producing a mixture of compound (II) and compound (III) from compound (I) and the method for producing a mixture of compound (IV) and compound (V) from compound (I)

25 of the present invention include a step of reacting compound (I) with a metal such as alkali metals (e.g., lithium, sodium, potassium etc.), alkaline earth metals (e.g., magnesium, calcium, strontium, barium etc.) and the like. Of these, alkali metals such as lithium, sodium, potassium and the like

30 are preferable, and lithium is more preferable.

The amount of these alkali metal or alkaline earth metal to be used is not particularly limited as long as the reduction of the carbon-carbon double bond at the 4,5-positions of compound (I) is almost completed and the carbon-

35 carbon double bond at the 1,2-positions of compound (I) can

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partly remain. However, in order to significantly suppress the reduction of ketone, the amount is generally within the range of 0.8 to 2.5 times the amount necessary for reducing the carbon-carbon double bond at the 4,5-positions of compound (I). When the amount of alkali metal or alkaline earth metal to be used is less than such range, reduction of the carbon-carbon double bond at the 4,5-positions of compound (I) tends not to be completed, and when it is greater than such range, side reactions (e.g., reduction of ketone and the like) tend to proceed.

The reaction temperature is preferably within the range of -100°C to 50°C, more preferably within the range of -50°C to 20°C. While the reaction time varies depending on the reaction conditions, it is preferably within the range of 0.1 to 20 hr, more preferably within the range of 1 to 10 hr, from the industrial viewpoints.

The reduction reaction is carried out in the presence of ammonia and/or an amine. The kind of amine is not particularly limited and, for example, linear, branched or cyclic amines having 1 to 6 carbon atoms such as primary amines (e.g., methylamine, ethylamine, isopropylamine, butylamine and the like); secondary amines (e.g., dimethylamine, diethylamine, diisopropylamine, pyrrolidine, piperidine and the like); polyamines (e.g., ethylenediamine, diaminopropane, N,N'-dimethylethylenediamine and the like); and the like can be mentioned, with preference given to ammonia.

The amount of the ammonia and/or amine to be used is preferably within the range of 1- to 100-fold by mass, more preferably within the range of 3- to 50-fold by mass, relative to compound (I).

In addition, use of a proton donor is necessary for the reaction. While the kind of the proton donor is not particularly limited and, for example, inorganic acids such as hydrochloric acid, sulfuric acid, carbonic acid and the like, carboxylic acids such as formic acid, acetic acid, benzoic

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acid and the like, ammonium salts or amine salts thereof;
water; alcohol and the like can be mentioned, with preference
given to alcohol. As the alcohol, for example, linear,
branched or cyclic alcohols having 1 to 12 carbon atoms such
as primary alcohols (e.g., methanol, ethanol, 1-propanol, 1-
butanol, 1-octanol, 1-dodecanol and the like); secondary
alcohols (e.g., 2-propanol, 2-butanol, 3-pentanol,
cyclopentanol, cyclohexanol, 2-octanol and the like); tertiary
alcohols (e.g., tert-butanol, tert-amylalcohol, 2-
methylhexanol, 1-methylcyclohexanol and the like); polyhydric
alcohols (e.g., ethylene glycol, 1,4-butanediol, 2,4-
pentanediol, glycerol and the like); and the like can be
mentioned. Of these, tertiary alcohol is preferable, and tert-
butanol is more preferable.

The amount of the proton donor to be used is generally
within the range of 1.5- to 3-fold by mol per one carbon-
carbon double bond to be reduced.

The timing of the addition of the proton donor to the
reaction system is not particularly limited and can be freely
selected from, for example, a method including addition of a
proton donor to the reaction system before reaction of
compound (I) with an alkali metal or alkaline earth metal, a
method including addition of a proton donor to the reaction
system after reaction of compound (I) with an alkali metal or
alkaline earth metal and the like, with preference given to
the former method.

The reduction reaction may be carried out in the presence
of a solvent. The usable solvents are not particularly limited
as long as they do not adversely influence the reaction and,
for example, ethers such as tetrahydrofuran, diethyl ether,
diisopropyl ether, methyl tert-butyl ether, cyclopentyl methyl
ether, dimethoxyethane, 1,4-dioxane and the like; saturated
aliphatic hydrocarbons such as pentane, hexane, heptane,
octane and the like; and the like can be mentioned. Of these,
ethers such as tetrahydrofuran, diethyl ether, diisopropyl

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ether, methyl tert-butyl ether, dimethoxyethane, 1,4-dioxane and the like are preferable, and tetrahydrofuran is more preferable.

When a solvent is used, its amount of use is not particularly limited. However, it is preferably within the range of 1- to 100-fold by mass, more preferably within the range of 3- to 50-fold by mass, relative to compound (I).

By such reduction reaction, compound (I) is stereoselectively reduced such that the hydrogen atom at the 5-position of pregnane has an α configuration. As used herein, by the stereoselectively is meant that compound (II) and compound (III), or compound (IV) and compound (V) are produced in greater amounts than isomers wherein the hydrogen atom at the 5-position of pregnane has a β configuration.

The method of isolation and purification of the product after the reduction reaction is not particularly limited, and methods generally used for the isolation and purification of organic compounds can be employed. For example, a mixture of compound (II) and compound (III), or a mixture of compound (IV) and compound (V) can be obtained by concentration after extraction operation and the like.

The hydroxyl-protecting group represented by R^1 or R^2 in compound (I) may be the same as or different from the hydroxyl-protecting group represented by R^{11} or R^{12} in a mixture of compound (II) and compound (III), or the hydroxyl-protecting group represented by R^{21} or R^{22} in a mixture of compound (IV) and compound (V). In other words, the hydroxyl-protecting group represented by R^1 or R^2 may optionally vary by carrying out a reduction reaction (Birch reduction reaction) as long as it can be deprotected. For example, a benzoyl group may change to a 2,5-cyclohexadienecarbonyl group as a result of a reduction reaction.

In addition, the hydroxyl-protecting group represented by R^1 or R^2 in compound (I) may be eliminated by carrying out a reduction reaction during a step for producing a mixture of

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compound (II) and compound (III) from compound (I).

3. Deprotection method of hydroxyl-protecting group and reaction conditions

The reaction conditions used for elimination of a hydroxyl-protecting group is not particularly limited, and those generally used depending on the kind of the protecting groups can be selected for use.

For example, in the case of a tri-substituted silyl group for which a hydroxyl-protecting group is preferable, a mixture of compound (II) and compound (III), or a mixture of compound (IV) and compound (V) is reacted with an acid or fluoride to allow deprotection. While this embodiment is explained in the following, the deprotection is not limited to the embodiment.

The kind of the acid is not particularly limited and, for example, inorganic acids such as hydrochloric acid, sulfuric acid, hydrofluoric acid, hydrobromic acid and the like; organic acids such as acetic acid, trifluoroacetic acid, p-toluenesulfonic acid, methanesulfonic acid and the like; and the like can be mentioned. As the fluorides, for example, tetrabutylammonium fluoride, potassium fluoride, sodium fluoride and the like can be mentioned.

The amount of the acid to be used is within the range of 0.01- to 10-fold by mol, more preferably within the range of 0.1- to 5-fold by mol, relative to the raw material.

The amount of the fluoride to be used is determined based on the number of the protecting groups contained in the raw material, which are to be eliminated. Preferably, it is within the range of 1- to 10-fold by mol, more preferably within the range of 1- to 5-fold by mol, relative to one protecting group.

The deprotection may be carried out in the presence of a solvent. Usable solvents are not particularly limited as long as they do not adversely influence the reaction and, for example, ethers such as tetrahydrofuran, diethyl ether, diisopropyl ether, methyl tert-butyl ether, cyclopentyl methyl

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ether, dimethoxyethane, 1,4-dioxane and the like; saturated aliphatic hydrocarbons such as pentane, hexane, heptane, octane and the like; and the like can be mentioned. Of these, ethers such as tetrahydrofuran, diethyl ether, diisopropyl ether, methyl tert-butyl ether, dimethoxyethane, 1,4-dioxane and the like are preferable, and tetrahydrofuran is more preferable.

When a solvent is to be used, its amount to be used is not particularly limited, but preferably within the range of 1- to 100-fold by mass, more preferably within the range of 3- to 50-fold by mass, relative to the raw material.

The reaction temperature is preferably within the range of -20°C to 120°C, more preferably within the range of 0°C to 80°C. The reaction time is preferably within the range of 0.1 to 20 hr, more preferably within the range of 1 to 10 hr.

The method of isolation and purification of the obtained product is not particularly limited, and methods generally used for the isolation and purification of organic compounds can be employed. For example, a mixture of compound (VI) and compound (VII) can be obtained by concentration after extraction operation and the like.

4. Reduction of a mixture of compound (II) and compound (III), or a mixture of compound (IV) and compound (V)

The compound (III) in a mixture of compound (II) and compound (III), or compound (V) in a mixture of compound (IV) and compound (V) can be converted to an objective saturated ketone by reducing the carbon-carbon double bond at the 1,2-positions. As the reduction method, for example, hydrogenation using a transition metal catalyst, reduction with a hydride agent, the aforementioned reduction method as a method for producing a mixture of compound (II) and compound (III) from compound (I), and the like can be mentioned. Of these, hydrogenation using a transition metal catalyst is preferable since reduction of ketone can be easily suppressed. While the hydrogenation using a transition metal catalyst, which is a

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preferable embodiment, is explained in the following, the reduction step is not limited to this embodiment.

The hydrogenation is carried out by reacting a mixture of compound (II) and compound (III), or a mixture of compound (IV) and compound (V) with a reducing agent in the presence of a transition metal catalyst.

As the metal of the transition metal catalyst to be used for the hydrogenation, for example, ruthenium, rhodium, iridium, nickel, palladium, platinum and the like can be mentioned. Of these, nickel, palladium and platinum are preferable, and palladium is most preferable. The form of the transition metal catalyst may be a complex catalyst (e.g., tetrakis(triphenylphosphine)palladium, palladium acetate) that dissolves in a reaction system, or a heterogeneous catalyst (for example, palladium-carbon, palladium hydroxide, palladium black, platinum oxide) that does not dissolve in a reaction system. However, a heterogeneous catalyst is preferable, because it is easily separated from the reaction system, and palladium-carbon and palladium black are particularly preferable.

While the amount of the transition metal catalyst to be used varies depending on the ratio of the compound (III) or compound (V) contained in a raw material mixture, it is generally within the range of 0.01 to 100% by mass, preferably within the range of 0.1 to 10% by mass, relative to the total mass of the raw material mixture.

As the reducing agent, molecular hydrogen, formic acid and a salt thereof and the like can be mentioned, with preference given to molecular hydrogen.

The hydrogen partial pressure when a molecular hydrogen is used as a reducing agent is preferably within the range of 1×10^4 to 1×10^7 Pa, more preferably within the range of 1×10^5 to 1×10^6 Pa.

The reaction temperature for the hydrogenation is preferably within the range of 0°C to 150°C, more preferably

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within the range of 20°C to 100°C. The reaction time varies depending on the reaction conditions, but preferably within the range of 0.1 to 20 hr, more preferably within the range of 1 to 10 hr, from the industrial viewpoints.

The hydrogenation reaction is generally carried out in the presence of a solvent. The solvent is not particularly limited as long as it does not adversely influence the reaction and, for example, ethers such as tetrahydrofuran, diethyl ether, diisopropyl ether, methyl tert-butyl ether, cyclopropyl methyl ether, dimethoxyethane, 1,4-dioxane and the like; saturated aliphatic hydrocarbons such as pentane, hexane, heptane, octane and the like; aromatic hydrocarbons such as benzene, toluene, xylene, mesitylene and the like; esters such as methyl acetate, ethyl acetate, butyl acetate, methyl benzoate and the like; alcohols such as methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 1-octanol and the like; nitriles such as acetonitrile and the like; amides such as N,N'-dimethylformamide, N-methylpyrrolidone and the like; dimethyl sulfoxide and the like can be mentioned, which may be used alone or in combination. Of these, ethers such as tetrahydrofuran, diethyl ether, diisopropyl ether, methyl tert-butyl ether, cyclopropyl methyl ether, dimethoxyethane, 1,4-dioxane and the like are preferable, and tetrahydrofuran is more preferable.

While the amount of the solvent to be used is not particularly limited, it is preferably within the range of 1- to 200-fold by mass, more preferably within the range of 3- to 50-fold by mass, relative to the total mass of the raw material mixture.

The method of isolation and purification of compound (II) or compound (IV) obtained by the reduction reaction is not particularly limited, and methods generally used for the isolation and purification of organic compounds can be employed. For example, compound (II) or compound (IV) can be isolated and purified by recrystallization or column

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chromatography after catalyst removal, extraction operation and the like.

5. Secure supply of raw material

The production method of compound (I) to be used as a raw material is not particularly limited. For example, (20S)-7α,21-dihydroxy-20-methylpregna-1,4-dien-3-one can be easily obtained by subjecting 3α,7α-dihydroxy-5β-cholanoic acid and/or a salt thereof to a conversion reaction using a microorganism (JP-B-2525049) to give 7α-hydroxy-3-oxo-pregna-1,4-diene-20α-carbaldehyde, and reducing the 20-position of the compound with sodium borohydride (WO02/20552), and (20S)-7α,21-dihydroxy-20-methylpregn-4-en-3-one can be easily obtained by subjecting 3α,7α-dihydroxy-5β-cholanoic acid to a conversion reaction using a microorganism to give 7α-hydroxy-3-oxo-pregn-4-ene-20α-carbaldehyde, and reducing the aldehyde group of the compound with sodium borohydride (WO03/23047). By protecting as necessary the hydroxyl groups at the 21-position and the 7-position of these compounds by a method known *per se*, compound (I) to be used in the present invention can be afforded.

Examples

The present invention is explained in more detail in the following by referring to Examples, which are not to be construed as limitative.

Reference Example 1

Production of (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methylpregna-1,4-dien-3-one

Under a nitrogen atmosphere, (20S)-7α,21-dihydroxy-20-methylpregna-1,4-dien-3-one (8.79 g, 25.5 mmol), imidazole (2.60 g, 38.3 mmol) and tetrahydrofuran (100 ml) were placed in a 200 ml flask, dissolved by stirring and ice-cooled. To this solution was added dropwise a solution of tert-butyldimethylchlorosilane (5.00 g, 33.2 mmol) dissolved in tetrahydrofuran (20 ml) while maintaining the inner temperature at 0°C to 10°C. After completion of the addition,

the mixture was allowed to warm to room temperature and further stirred for 1 hr. The reaction solution was added to water (200 ml) and the mixture was extracted twice with ethyl acetate (100 ml). The aqueous layer was separated, and the organic layer was washed with saturated brine (100 ml), dried over anhydrous sodium sulfate and concentrated. The obtained crude product was purified by silica gel column chromatography to give (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methylpregna-1,4-dien-3-one (11.11 g, yield 95%) having the following property.

^{1}H-NMR spectrum (270MHz, CDCl$_3$, TMS, ppm) δ: 0.03(s, 6H), 0.76(s, 3H), 0.89(s, 9H), 0.99(d, 3H, J=6.9Hz), 1.1-1.8(15H), 2.03(dt, 1H, J=3.0, 12.9Hz), 2.48(dd, 1H, J=3.0, 13.9Hz), 2.75(dt, 1H, J=2.0, 13.9Hz), 3.28(dd, 1H, J=6.9, 9.9Hz), 3.56(dd, 1H, J=3.0, 9.9Hz), 4.05(bs, 1H), 6.14(dd, 1H, J=0.9, 2.0Hz), 6.24(dd, 1H, J=2.0, 9.9Hz), 7.08(d, 1H, J=9.9Hz).

Example 1

Production of a mixture of (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-3-one and (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-1-en-3-one

Under a nitrogen atmosphere, tetrahydrofuran (85 ml), (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methylpregna-1,4-dien-3-one (5.00 g, 10.9 mmol) and tert-butanol (3.47 g, 46.8 mmol) were placed in a 300 ml three-necked flask. The mixture was cooled to below -50°C and liquid ammonia (85 ml) was added. Then, metal lithium (0.32 g, 46.1 mmol) was slowly added while maintaining the inner temperature at -50°C to -40°C. After completion of the addition, the mixture was further stirred at -40°C for 2 hr. Ammonium acetate (3.61 g, 46.8 mmol) was added to the reaction solution, and the mixture was stirred for 12 hr to remove ammonia while allowing the reaction mixture to warm to room temperature. To the obtained tetrahydrofuran solution was added 15% by mass of an aqueous sulfuric acid solution to

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adjust the pH of the aqueous layer to 4 to 6, and the organic layer was separated from the aqueous layer. The organic layer was analyzed by HPLC and found to contain (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-3-one (3.81 g, yield 76%) and (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-1-en-3-one (0.65 g, yield 13%). HPLC conditions   column: TSK-gel80TM, inner diameter 4.6 mm x length 250 mm, column temperature: 35°C, mobile phase: acetonitrile/water=9/1, flow rate: 1 ml/min, detection wavelength: 210 nm, retention time: (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-3-one = 30 min, (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-1-en-3-one = 24 min

Example 2

Production of a mixture of (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one and (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-1-en-3-one

Under a nitrogen atmosphere, a tetrahydrofuran solution containing (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-3-one (3.81 g, 8.2 mmol) and (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-1-en-3-one (0.65 g, 1.4 mmol), obtained in Example 1, was placed in a 200 ml three-necked flask, then 6N hydrochloric acid (2 ml) was added, and the mixture was stirred at 40°C for 2 hr. After confirmation of the disappearance of the raw material by TLC, the reaction solution was allowed to cool to room temperature. The aqueous layer was adjusted to pH 8 with 10% by mass of an aqueous sodium hydroxide solution, and the organic layer was separated. The organic layer was analyzed by HPLC and found to contain (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one (2.76 g, yield 96%) and (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-1-en-3-one (0.46 g, yield 95%). HPLC conditions   column: Vydac218TP54 300A5u, inner diameter 4.6 mm x length 250 mm, column temperature: 35°C, mobile phase: acetonitrile/water=3/7, flow rate: 1 ml/min, detection

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wavelength: 210 nm, retention time: (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one = 30 min, (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-1-en-3-one = 24 min

Reference Example 2

Production of (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one

Under a nitrogen atmosphere, a tetrahydrofuran solution containing (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one (2.76 g, 7.9 mmol) and (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-1-en-3-one (0.46 g, 1.3 mmol), obtained in Example 2, was placed in a 200 ml three-necked flask, 10% palladium-carbon (50 mg) was added thereto and, after substitution with a hydrogen atmosphere, the mixture was reacted at 50°C under an atmospheric pressure for 5 hr. After confirmation of the disappearance of (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-1-en-3-one by HPLC analysis, the reaction mixture was allowed to cool to room temperature. The catalyst was filtered off and the filtrate was concentrated. The obtained crude product was purified by silica gel column chromatography to give (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one (3.06 g) having the following properties {yield 95% (based on the total of (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one contained and (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-1-en-3-one)}.

^{1}H-NMR spectrum (270MHz, CDCl$_3$, TMS, ppm) δ: 0.71(s, 3H), 1.01(s, 3H), 1.04(d, 3H, J=6.9Hz), 1.0-2.5(22H), 3.34(dd, 1H, J=6.9, 10.9Hz), 3.61(dd, 1H, J=3.0, 10.9Hz), 3.84-3.85(brs, 1H).

Reference Example 3

Production of (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-3-one

Under a nitrogen atmosphere, a tetrahydrofuran solution containing (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-3-one (4.09 g, 8.8 mmol) and (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-1-en-3-one (0.52 g, 1.1 mmol), obtained by the operation similar to that in Example 1, was placed in a 200 ml three-necked flask, 10%

palladium-carbon (50 mg) was added thereto and, after substitution with a hydrogen atmosphere, the mixture was reacted at 50°C under an atmospheric pressure for 5 hr. After confirmation of the disappearance of (20S)-21-tert-

5 butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-1-en-3-one by HPLC analysis, the reaction mixture was allowed to cool to room temperature and the catalyst was filtered off. The filtrate was analyzed by HPLC and found to contain (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-3-one

10 (4.47 g) {yield 97% (based on total of (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-3-one contained and (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-1-en-3-one)}.

Reference Example 4

15 Production of (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one

Under a nitrogen atmosphere, a tetrahydrofuran solution containing (20S)-21-tert-butyldimethylsilyloxy-7α-hydroxy-20-methyl-5α-pregn-3-one (4.47 g, 9.7 mmol), obtained in Reference Example 3, and 6N hydrochloric acid (2 ml) were

20 placed in a 200 ml three-necked flask, and the mixture was stirred at 40°C for 2 hr. After confirmation of the disappearance of the raw material by TLC, the reaction solution was allowed to cool to room temperature. The aqueous layer was adjusted to pH 8 with 10% by mass of an aqueous

25 sodium hydroxide solution, and the organic layer was separated and concentrated. The obtained crude product was purified by silica gel column chromatography to give (20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one (3.15 g, yield 94%).

Industrial Applicability

30 Compound (II), compound (IV) and compound (VI) ((20S)-7α,21-dihydroxy-20-methyl-5α-pregn-3-one) produced by the present invention can be easily converted to squalamine by the method described in WO01/79255. Therefore, the method of the present invention can be advantageously used for the

35 production of synthetic intermediates for squalamine.

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This application is based on patent application No. 108434/2004 filed in Japan on March 31, 2004, the contents of which are hereby incorporated by reference.

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